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The Role of Tumor Associated Macrophage in Recurrent Growth of Tumor Stem
Cell

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14. ABSTRACT: The pro-inflammatory microenvironment in the tumor is established by first recruiting various leukocytes including tumor-associated macrophage (TAM) which is believed to promote tumor growth and angiogenesis, suppress adaptive immunity, and hence remodel tumor microenvironment. According to the recent cancer stem cell (CSC) theory, recurrent tumor must arise from a dormant tumor stem cell whose re-growth is triggered by shifting of microenvironment. This project aims at clarifying the roles of TAM in recurrent growth of dormant stem cell in breast cancer. We hypothesize that the balance of dormancy and recurrence is determined by the ability of the tumor stem cells to recruit TAM which in turn promotes self-renewal of the stem cell. We have established necessary mouse colonies and also developed the method to generate TAM. We have also shown that TAM indeed promotes the growth of CSCs in our animal model. In the next fiscal year, we will address two important points. First, we will clarify whether TAM indeed plays a critical role in recurrent growth of tumor stem cells, which we believe will bring in a paradigm shift into our research field for the understanding of the mechanism of recurrent tumor growth. Secondly, the results of this research will provide us with strong rationale for targeting TAM for chemo-prevention of recurrent breast cancer.					
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INTRODUCTION

Tumor cells often induce inflammatory responses and mobilize a variety of leukocytes around the tumor region, which is considered as a normal host-defense mechanism to block cancer growth. Interestingly, the risk of breast cancer recurrence has been shown to be significantly decreased by taking anti-inflammatory drugs, suggesting that inflammation plays a critical role in recurrence (1). Although TAM is one of these inflammatory responding cells, it closely resembles M2 (alternative) phenotype which exhibits anti-inflammatory and tumor-promoting functions (2). It has been also suggested that TAM provides favorable microenvironment for tumor stem cell growth by generating a suitable niche (3). According to the recent cancer stem cell theory, which still remains a hypotheses, recurrent tumor must arise from a dormant tumor stem cell whose re-growth is triggered by shifting of microenvironment. This project aims at clarifying the roles of TAM in recurrent growth of dormant stem cell in breast cancer. We hypothesize that the balance of dormancy and recurrence is determined by the ability of the tumor stem cells to recruit TAM which in turn promotes self-renewal of the stem cell.

BODY

Task 1. To examine whether reciprocal interaction of tumor stem cells and tumor-associated macrophage (TAM) can promote the stem cell growth

- Isolate tumor stem cell from primary and recurrent cancer in recurrent mouse model
- Test whether conditioned media of tumor stem cells activates macrophage
- Examine whether TAM promotes growth of tumor stem cells

To accomplish Task 1(a), we have obtained MMTV-rtTA and TetO-ErbB2 mice and established both the colonies of MMTV-rtTA^{+/-} and TetO-ErbB2^{+/-}. We have also cross-bred these animals and

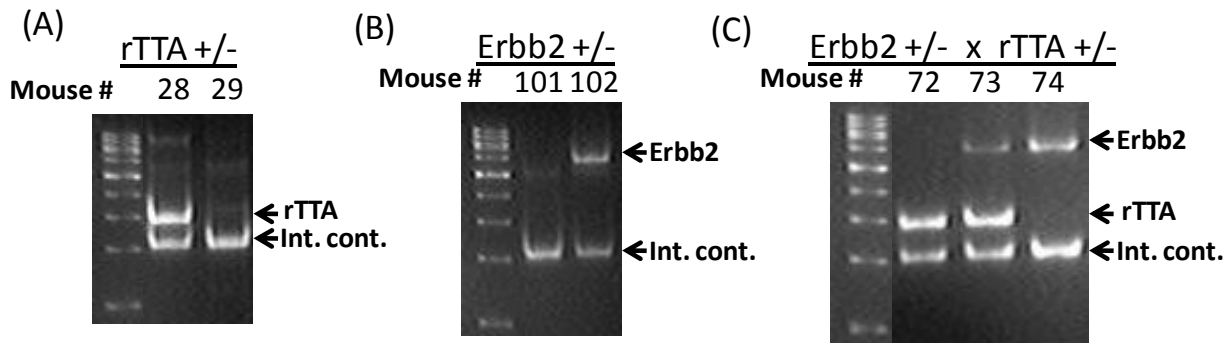
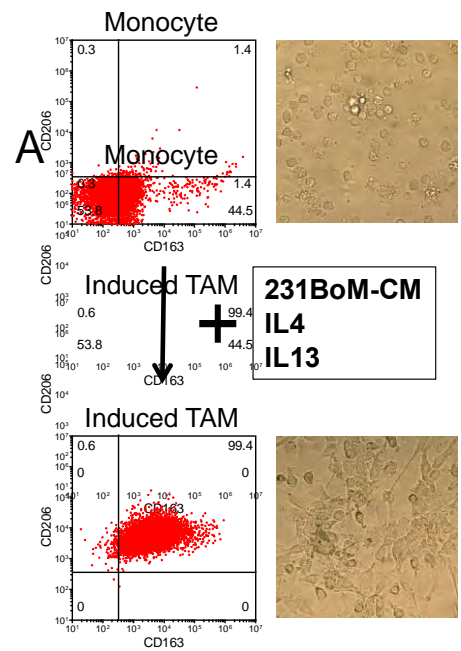


Fig. 1. Ongoing mouse colony breeding. (A) Example of genotypes of ongoing colony breeding for MMTV-rtTA. #28 mouse has rtTA transgene. (B) Example of genotypes of ongoing colony breeding for TetO-ErbB2. #102 mouse has ErbB2 transgene. (C) Example of genotypes of ongoing colony breeding for the hybrid of MMTV-rtTA and TetO-ErbB2. #72 has only rtTA, #73 has only ErbB2 and #74 has both rtTA and ErbB2 transgenes.

obtained MMTV-rtTA^{+/-} /TetO-ErbB2^{+/-} heterozygous animals (**Fig. 1**). These mice were not lethal without feeding Doxocycline, and they can serve for our experiments. However, this cross-breeding is quite inefficient, and therefore, we are currently trying to establish homozygous mouse for both genes (MMTV-rtTA^{+/+}, and TetO-ErbB2^{+/+}). If successful, the cross-breeding of these mice will yield desired phenotype of offspring with 100% efficiency. Therefore, Task 1(a) is still ongoing. In Aim (b), to establish the method for generating tumor associated macrophage (TAM), we have isolated human monocytes and treated them with IL4, IL13 and conditioned medium of human breast cancer

cell, MDA-MB231BoM, which preferentially metastasizes to the bone in our animal model. As shown in **Fig. 2**, this treatment of monocytes with these three components made significant changes to the morphology of macrophages and became adherent to plastic dish. While we are waiting for the progress of Task 1(a), we tested the effect of TAM on the growth of cancer stem cell in an animal



model in order to accomplish Task 1(c). To examine the effect of TAMs on the growth of CSCs in the bone microenvironment, we co-injected TAM and CSCs of 231BoM directly into the tibial bones of mice followed by measuring bioluminescence for tumor growth. As shown in **Fig. 3**, the co-injection of TAMs with CSCs significantly augmented the tumor growth in the tibiae compared to the injection of CSCs alone, which strongly supports the idea that TAM plays a critical role in growth of CSCs in breast cancer. Therefore, we now have established the system to conduct a similar experiment using the primary and recurrent cancer cells when we accomplish Task 1(a). Therefore, Task 1 (b) is accomplished and Task 1 (c) is still ongoing.

Fig. 2. Preparation of TAM from monocytes. Primary human monocytes were treated with IL-4, IL-13 and conditioned medium of 231BoM for 7 days. A portion of the cells was analyzed by FACS using anti-CD163 and anti-CD206 antibodies. The morphology of the cells at day 7 is also shown.

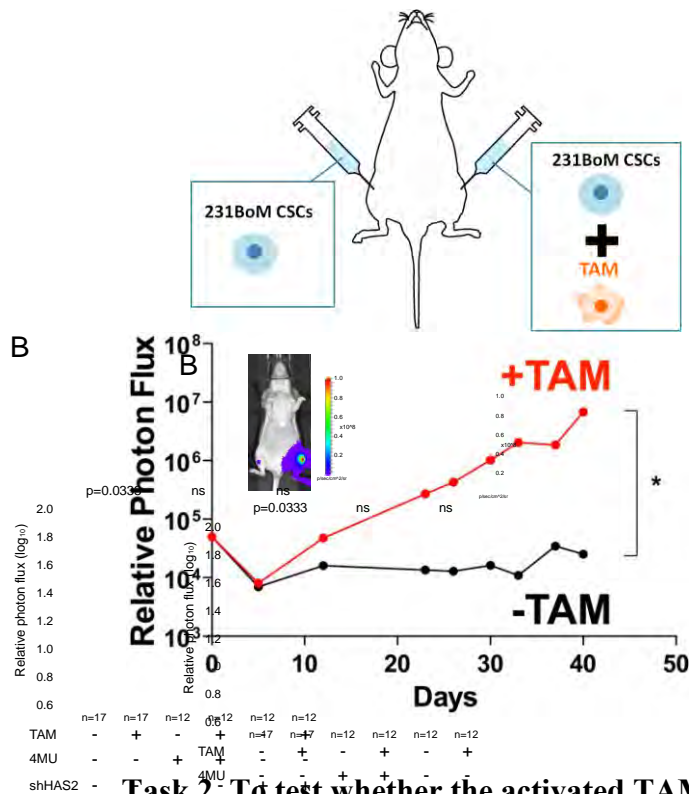


Fig. 3. Effect of TAM on the growth of CSC. CSCs from 231BoM were co-injected with (right tibia) or without (left tibia) TAMs in the same animals. The growth of tumor was then periodically measured by BLI.

Task 2: To test whether the activated TAM can promote recurrence in the animal model

- Examine the localization of TAM in recurrent mouse model.
- Test whether TAM induces recurrence of dormant breast cancer cells *in vivo*.

As described in Task 1, we are still in the process of cross-breeding MMTV-rtTA and TetO-ErbB2.

We expect that we will obtain these mice in 4 months. We can then conduct the experiment of Task 2 (a) and (b). However, as we described in the progress of Task 1, we have now a workable model system for testing the effect of TAM in bone. Therefore, we hope we can accomplish Task 2 in the next fiscal year. To this purpose, we requested no-cost extension of this grant and it was approved.

KEY RESEARCH ACCOMPLISHMENTS

1. We have established mouse colonies for MMTV-rtTA and TetO-ErbB2. The cross-breeding is ongoing.
2. We have established the methodology of generating tumor associated macrophage from monocytes.
3. We found that TAM significantly promotes the growth of CSCs in the bone in our animal model.

REPORTABLE OUTCOMES

Peer reviewed publications

None

Abstract/presentation

1. Aya Kobayashi, Hiroshi Okuda, Fei Xing, Wen Liu, Puspa Pandey, Misako Watabe, Sudha K. Pai, and Kounosuke Watabe. The role of tumor associated macrophage in recurrent growth of tumor stem cell in bone metastasis. Department of Defense (DOD) Breast Cancer Research Program (BCRP) Era of Hope 2011 Conference. Orlando, Florida

Employment

1. Aya Kobayashi, Ph.D. (postdoctoral fellow) has been partly supported by the current grant.
2. Mr. Vishnu Modur (Graduate student) has been partly supported by the grant.

CONCLUSIONS

We have established necessary mouse colonies and also developed the method to generate TAM. We have also shown that TAM indeed promotes the growth of CSCs in our animal model. Therefore, we are now in a strong position to pursue rest of the tasks. In the next fiscal year, we will address two important points. First, we will clarify whether TAM indeed plays a critical role in recurrent growth of tumor stem cells, which we believe will bring in a paradigm shift into our research field for the understanding of the mechanism of recurrent tumor growth. Secondly, the results of this research will provide us with strong rationale for targeting TAM for chemo-prevention of recurrent breast cancer.

So what?

The pro-inflammatory microenvironment in the tumor is established by first recruiting various leukocytes including tumor-associated macrophage (TAM) which is believed to promote tumor growth and angiogenesis, suppress adaptive immunity, and hence remodel tumor microenvironment (4). Therefore, understanding the underlining mechanism of tumor-TAM interaction in cancer recurrence is of paramount interest for developing a novel approach to treat and prevent recurrent breast cancer. We believe that the outcome of our results will clarify the role of TAM in tumor recurrence and identify possible target for the treatment of recurrent breast cancer.

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